CITRUS

RESEARCH CONFERENCE

January 24, 1956

Fruit and Vegetable Chemistry Laboratory

Pasadena 5, California

Western Utilization Research Branch

Agricultural Research Service

UNITED STATES DEPARTMENT OF AGRICULTURE

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PROGRAM AND ABSTRACTS OF PAPERS

CITRUS RESEARCH CONFERENCE

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Fruit and Vegetable Chemistry Laboratory
263 South Chester Avenue
Pasadena, 5, California

FOREWORD

This Citrus Research Conference is being held to bring to members of the citrus and allied industries in California and Arizona the latest results of research work on the chemistry and technology of citrus fruits and fruit products carried on by laboratories of the Agricultural Research Service, U. S. Department of Agriculture, and by cooperating laboratories. Some of the results of work herein presented are preliminary in nature, while others may represent various stages of advancement or even final results.

The following research agencies are participating:

U. S. Department of Agriculture, Agricultural Research Service

Western Utilization Research Branch
Western Regional Research Laboratory (Branch Headquarters), Albany, California
Fruit and Vegetable Chemistry, Pasadena, Calif.

Southern Utilization Research Branch
Citrus Products Station, Winter Haven, Fla.
Fruit and Vegetable Products Laboratory, Weslaco, Texas

University of California, Agricultural Experiment Station
Citrus Experiment Station, Riverside, Calif.

Continental Can Company, Chicago, Ill.

P R O G R A M ---- C I T R U S R E S E A R C H C O N F E R E N C E

January 24, 1956 ----- 10 AM to 5 PM

(Names of those who will present papers are shown here.
Complete authorships are shown on later pages.)

A PROGRESS REPORT ON THE CHRONIC TOXICITY OF BIPHENYL

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Western Regional Research Laboratory
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The chronic toxicity study on biphenyl was started with 15 albino weanling rats of each sex on the Addis diet containing 0.0, 0.001, 0.005, 0.01, 0.05, 0.10, 0.50, and 1.0 percent biphenyl. Since composition of the basal diet sometimes alters the toxicity picture, a similar experiment was started about 50 days later with the commercial Purina diet. Shifting the decimal point 4 places to the right expresses the dosage levels in terms of parts of biphenyl per million parts of diet. The dosage range extends from 10 to 10,000 parts per million.

At the present time, these feeding tests have been in progress about 600 days with the Addis diet and 550 days with the Purina diet. Effects on growth rate have had little significance after 200 days, but during this period the food intake and rate of growth were lower on the 0.5 and 1.0 percent levels of biphenyl than on the control diets.

For all practical purposes an increased mortality rate as compared with the controls appears to be limited to the three highest dosage levels: namely, 0.1, 0.5, and 1.0 percent biphenyl. There is a slight suggestion that the mortality rate on the two higher dosages has been greater and has started earlier on the Purina diet than on the Addis diet. A total of 7 tumors have been noted to date. They are all in the females and probably unrelated to the biphenyl intake, because of their small number, lack of relationship to dosage level, and absence in rats on the 1.0 percent dosage level.

No conclusions can be drawn at present regarding the potential hazards or margin of safety in the use of biphenyl. The two-year feeding period is still in progress. Following its completion, appreciable time will be required to autopsy the hundreds of animals, prepare stained tissue sections of all organs and make detailed examination for evidence of damage.

HEAT STABILIZATION IN THE PREPARATION OF FROZEN CONCENTRATED ORANGE JUICE

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and
R. B. Guyer and W. M. Miller
Continental Can Company, Inc., Chicago, Illinois

During the past three years the U. S. Citrus Products Station and the Continental Can Company, Inc., have cooperated in an extensive citrus research program at the Winter Haven Station. Investigations included: (1) heat treatments at different temperatures for several intervals of time; (2) a comparison of several methods of heat stabilization; (3) a study of seasonal and varietal factors as related to heat stabilization; (4) effect of heat treatment at intermediate levels of concentration; and (5) production and stability of high-Brix concentrate.

In the first series of experiments a small tube heat exchanger was used to give a wide range of treatment temperatures and holding times. Heating at 150°F. for 15 seconds or 160°F. for 5 seconds reduced the pectinesterase (PE) activity 75 percent or more. Little benefit was found in heating for more than 15 seconds with the exception of 140°F. treatment. The PE activity was greatly reduced with increasing treatment temperatures in the range of 140° to 170°F. but cloud stability was not proportional. Further increase in treatment temperature in the range of 170° to 180°F. effected relatively small further reductions in PE activity but gave a pronounced increase in cloud stability.

In the second portion of the investigation steam injection was more efficient in inactivating PE at 150°F. than either the small-tube or plate type pasteurizers. With higher treatment temperatures there was little difference in residual PE, yet the stability of the steam-injection product was as good as, or better than, that of either of the other heat exchangers.

The third portion was concerned with seasonal and varietal factors as related to heat stabilization. Heat treatment at 150° and 160°F. was more effective in inactivating PE in Valencia juice and least in Hamlin juice. Hamlin and Pineapple varieties showed little effect of maturity on enzyme inactivation and yet treatment at 150° and 160°F. was somewhat more effective with early Valencia juice than with late Valencia juice. Similar trends were noted for cloud stability. Seasonal and varietal factors are being investigated further during the 1955-56 season.

In the fourth portion the effect of application of heat at intermediate levels of concentration was studied. This study is not complete, but so far, no appreciable difference in residual enzyme activity due to the concentration has been noted. However, the cloud was more stable than when heat was applied to the single-strength juice.

The fifth portion was concerned with the production and stability of high-Brix (58.5°) concentrate. These products were inherently more stable than comparable 42° Brix products. The 42° Brix concentrates of 150°F. treatment were equivalent in cloud stability to 58.5° Brix controls of no heat treatment, and the 150°F. treatment in preparation of the high-Brix product gave complete cloud stability for at least 42 days. In processing high-Brix concentrates only half the evaporator feed juice need be heated to 150°F. to insure complete cloud stability for at least 42 days.

When evaporator products of 62°, 64°, and 66° Brix which had received 150°F. treatment were cut back with unheated juice to 58.5° Brix, all products over the entire range of concentration levels and treatment temperatures were stable for at least 42 days.

There were two instances in which the taste panel noted flavor differences. Concentrate prepared from late Valencia juice was considered more desirable than that from earlier Valencia fruit. In late-season pineapple-orange concentrates there were definite indications that heat treatments of 170° and 180°F. affected the flavor.

FURTHER STUDIES ON THE MECHANISMS OF CLOUD LOSS IN FROZEN CITRUS CONCENTRATES

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Studies of the mechanisms of cloud loss in frozen orange concentrates have been continued. These studies indicate that oxidative systems in citrus fruits and unheated orange concentrates play an important role in cloud-loss phenomena.

Commercially packed frozen orange concentrates are saturated with air. Manometric studies show that in the presence of oxygen unheated orange concentrates carry on a slow but significant respiration. Therefore, oxidative reactions are certain to occur in orange concentrates at elevated temperatures and may affect flavor and nutritive value as well as cloud.

In one experimental pack so far studied, it was found that cloud is significantly more stable in concentrate packed under high vacuum than in the same concentrate packed at atmospheric pressure after air had been mechanically whipped into it. The lot of concentrate used in these studies had, however, an exceptionally high inherent cloud stability for its pectinesterase (PE) content, and further studies with additional lots would be necessary to establish any real advantage of vacuum packing.

Studies of oxidative systems that could lead to degradation of pectic substances have shown that, under the conditions of pH and the sugar and acid content of orange concentrates, pectin is rapidly degraded in the presence of ascorbic acid and peroxide. Ascorbic acid is, of course, present in citrus products and preliminary work indicates that traces of peroxides may also be present. The possible role of this oxidative pathway of pectin degradation in cloud loss is under study.

Studies are continuing on the acceleration and inhibition of cloud loss by various chemical agents. Acceleration of cloud loss in orange concentrates by added peroxide appears to be related to a heat-labile enzyme system. Relatively large amounts of the enzyme catalase have been found in unheated orange concentrates. Its activity is associated almost exclusively with the insoluble-solids portions of the concentrate; little or no activity is found in the clear, filtered serum. The relationahip of this enzyme to cloud stability is obscure at present.

Fumaric acid, a naturally occurring constituent of living cells, is the most effective inhibitor of cloud loss in orange concentrates so far discovered. Both fumaric acid and its sodium salts have been found to

bring about a 50% or greater inhibition of cloud loss in California orange concentrates at the 0.025 to 0.05 molar level. Combinations of sodium fumarate with mild heat treatments are very effective in stabilizing orange concentrate.

In spite of repeated attempts, no relationship has been found between fumarate and PE activation or inactivation, nor has it been possible to establish any interaction of fumaric acid and pectic substances. This failure casts further doubt on the role of PE in cloud loss.

Publication: Properties of Stored Frozen Orange Concentrate Observed by Differential Cloud Determination. R. J. McColloch and R. G. Rice, Food Technol. 9, 70 (1955).

IDENTIFICATION OF THE CONSTITUENTS OF LEMON OIL

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During the past year work was initiated at this laboratory on the chemical composition of commercial lemon oils. The purpose was to apply recently developed chromatographic techniques to determine their chemical composition; to study processing, seasonal, and geographic variations and differences in maturity affecting the composition of lemon oils; and to establish the compositional identity of genuine oils.

Conventional adsorption chromatographic techniques on silicic acid developed in this laboratory by Dr. J. G. Kirchner for the study of the volatile constituents of grapefruit and orange juices were employed to obtain an initial separation of cold-pressed California lemon oils into 12 to 15 fractions homogeneous to silicic acid adsorption, but probably in many instances containing more than one component. These fractions will be further separated by vacuum distillation or by vapor-phase chromatography.

From the fractions obtained by chromatography on columns of powdered silicic acid 4 solid compounds have been isolated. These compounds are believed to contain the coumarin nucleus. They impart a blue fluorescence to lemon oil when it is exposed to ultraviolet light and are responsible for the characteristic spectral absorption peak of lemon oil appearing at about 315 millimicrons; on this absorption is based a recent A.O.A.C. method for characterizing lemon oils.

Two of these compounds give an intense blue fluorescence under ultraviolet light: one, limettin (5,7-dimethoxycoumarin) has been previously reported to be in lemon oil and dried lemon peel; the other, 7-methoxy-5-geranoxycoumarin, has been reported in West Indian lime oil and oil of bergamot.

The other two solid compounds do not fluoresce under ultraviolet light. From carbon and hydrogen analyses, ultraviolet absorption spectra, cleavage with weak acids, and behavior in the vanillin-hydrochloric acid test for phloroglucinol, these compounds have been tentatively assigned the furocoumarin structure. They are thought to be bergamottin, previously reported to be in oil of bergamot, and the hitherto unreported structural isomer of bergamottin which we have given the name iso-bergamottin. The four solids constitute a consistent series based on the biological precursor phloroglucinol (1,3,5-trihydroxybenzene).

A quantitative method of analysis using "chromatostrip" techniques is being used to determine the amounts of the blue-fluorescing compounds in lemon oil. Analyses of samples of lemon oil are under way to determine the pattern of their occurrence as governed by processing, seasonal, maturative, and geographic factors. This method could be applied, though not so conveniently, to the two nonfluorescing compounds. These compounds were found to be absent in distilled lemon oil, and the oils of bitter orange, sweet orange, and grapefruit. Although present in lime oil they are in extremely different and easily recognizable relative proportions.

We have also obtained evidence that at least 4 aldehydes are present in lemon oil, one of which is probably either decanal or dodecanal.

CHANGES IN THE CAROTENOID PIGMENTS OF VALENCIA ORANGE JUICE DURING CONCENTRATION, POWDER PREPARATION AND STORAGE THEREOF

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The off-flavor which develops in canned orange juice or powder on prolonged storage may originate, at least in part, in the carotenoid pigments. The carotenoids of California Valencia orange juice were previously investigated. Countercurrent distribution in a glass Craig apparatus was employed to separate the carotenoids into 6 fractions--hydrocarbons, monols, diols, monoether diols, diether diols, and polyols. These fractions were then resolved by chromatography into 26 components, not counting cis-isomers which may be artifacts. Five of these contained epoxide groups, 9 furanoxide groups, and 2 both groups. These cyclic ethers amount to over half of the carotenoid content of orange juice.

The carotenoids were investigated in a similar manner in samples of frozen single-strength and concentrated juices prepared from Florida Valencia oranges. The total carotenoid content of the Florida Valencia juice was somewhat lower than that of California Valencia juice; however, the compositions of the pigment mixtures were similar. Three minor constituents were found in Florida juice which have not yet been found in California juice. There was no significant difference between the composition of the carotenoid mixtures from the frozen single-strength and concentrated Florida juices.

Powdered juice was prepared by the puff-drying process from the concentrate to which had been added additional suspended material (which includes the carotenoid pigments) from single-strength juice. Examination of the carotenoid fraction of the powder showed that a minor part of the carotenoid epoxides had been isomerized to furanoxides. This acid-catalyzed isomerization results in paling of the color, since the furanoxides absorb light at shorter wavelengths than the corresponding epoxides. There was no apparent change in the non-ether carotenoids.

The powdered juice was conditioned for 78 days at 77°F. in order to reduce the moisture content to the desired level. Examination of the carotenoids at this time showed that over half of the epoxides had been converted to the isomeric furanoxides. There were no significant changes in the non-ether carotenoids. On storage of the conditioned powder for 6 months at 100°F., a loss of about 15 percent of the total carotenoids occurred. However, no loss occurred in the provitamin A carotenoids (α- and β-carotenes and cryptoxanthine). Two others (phytoene and phytofluene) likewise showed no losses. A loss of 10 to 27 percent was suffered by certain of the carotenoids (zeta-carotene, hydroxy-α-carotene, lutein, and zeaxanthine). On the other hand the epoxide

carotenoids had entirely disappeared. While the corresponding furanoxides increased, this increase was much less than the epoxide decrease, thus suggesting that some other change had occurred. The carotenoid epoxides are mild oxidizing agents. Reactions involving this property may result in substances causing off-flavor, either from the carotenoids themselves or from the accompanying lipides.

Publications

A. L. Curl. Application of Countercurrent Distribution to Valencia Orange Juice Carotenoids. J. Agr. and Food Chem., 1, 456 (1953).

A. L. Curl and G. F. Bailey. Polyoxygen Carotenoids of Valencia Orange Juice. J. Agr. and Food Chem., 2, 685 (1954).

A. L. Curl and G. F. Bailey. The State of Combination of the Carotenoids of Valencia Orange Juice. Food Research, 20, 371 (1955).

A. L. Curl and G. F. Bailey. The Carotenoids of Aged Canned Valencia Orange Juice. J. Agr. and Food Chem., in press.

A. L. Curl and G. F. Bailey. The Carotenoids of Valencia Orange Peel and Pulp. J. Agr. and Food Chem., in press.

IDENTIFICATION OF THE FLAVONOID CONSTITUENTS OF THE LEMON

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The flavonoid constituents of citrus fruits, particularly the lemon, have been the subject of numerous studies in recent years. Much of the uncertainty regarding the therapeutic properties of various flavonoid preparations stems from the fact that compounds of doubtful identity have often been used. A more detailed knowledge of the flavonoids of lemon is desirable, not only because of their use in therapy, but also because they have been implicated as possible substrates involved in the darkening of processed lemon juice.

Experiments have been undertaken to identify flavonoids present in the lemon and to isolate them in amounts large enough for further study, particularly of their physiological effects. The general procedure has been to extract dried, powdered lemon peel with successive portions of petroleum ether, ether, acetone, and methanol. The main portion of flavonoid glycosides is contained in the methanol extract, although a smaller quantity is also found in the acetone fraction.

Examination of the extracts by paper chromatography shows the presence of about five principal flavones and flavanones (one of which is hesperidin), although a number of others are present in smaller quantity. One of the flavones present in large amount has been isolated and identified as diosmin (3',5,7-trihydroxy-4'-methoxyflavone 7-rhamnoglucoside). It is estimated to comprise roughly 0.3-0.5% of the weight of the dried peel and crystallizes from the extracts together with hesperidin. It may be separated from the latter compound by prolonged heating with methanolic hydrochloric acid, which hydrolyzes and dissolves the hesperidin but does not affect the extremely insoluble diosmin. The identity of the diosmin was proved by comparing the melting point, R_f values and absorption spectrum with those of an authentic sample of the compound prepared by the oxidation of hesperidin. In addition, the compound was hydrolyzed to diosmetin, rhamnose and glucose, all of which were characterized. Examination of an extract of Valencia oranges failed to show the presence of diosmin in significant quantity.

During the course of this work a color reaction was found which appears to be specific for detecting flavanones in the presence of other flavonoids. The test, which may be applied either in solution or on paper chromatograms, depends on the production of a purple color when the flavanone is reduced with sodium borohydride and treated with acid. Flavonoids other than flavanones give a yellow color visible in ultraviolet light.

PHYSIOLOGICAL EFFECTS AND METABOLISM OF CITRUS FLAVONOIDS

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Vitamin P is the term given by Szent-Györgyi in 1936 to the material in a citrus fraction that is capable of clearing up the hemorrhagic condition in scurvy. It is a well-established fact that ascorbic acid (vitamin C) deficiency is the cause of scurvy, but Szent-Györgyi and coworkers claimed that administration of ascorbic acid to scorbutic guinea pigs did not remove residual hemorrhagic symptoms without simultaneous administration of the citrus fraction. Since the cutaneous hemorrhages observed in scurvy were presumed to be associated with an increased permeability of the cutaneous capillaries, the citrus factor visualized as decreasing the permeability was named vitamin P.

Vitamin P lacks the characteristics of a true vitamin. The naturally occurring product is not a single chemical entity. A state of vitamin P deficiency has never been produced experimentally in the presence of an adequate intake of recognized nutrients. More correctly speaking, the vitamin P concept includes a group of pharmacological properties and physiological effects possessed in varying degrees by a group of chemically related compounds known as flavonoids. The flavonoids are widely distributed in the plant kingdom. They are found in our fruits and vegetables. The hesperidin of oranges, the naringin of grapefruit, the diosmin and possibly eriodictyol of lemons are a few of the better known flavonoids in citrus fruits.

Since the original announcement of so-called "vitamin P" in 1936, an extensive literature of approximately 1000 references has developed. This literature, which is sometimes contradictory and difficult to evaluate, reports studies involving experimental animals under a variety of conditions, but the bulk of it concerns clinical use of flavonoids in many disease states directly or indirectly associated with bleeding tendency or abnormal functioning of the capillaries. In an effort to establish a background of reproducible findings on experimental animals under controlled conditions, studies with flavonoids have been made on the protective action toward epinephrine, the effect on cutaneous capillaries subjected to local irritation, the effect in acute cold injury and the metabolic fate after oral administration to animals.

In general, the flavonoids retard the oxidation of epinephrine and ascorbic acid, decrease the dilatation of the capillary network following irritation, decrease the severity of cold injury and are metabolized to various phenolic acids--phenyl propionic acids in the case of the citrus flavonoids studied to date.

EFFECTS OF PROCESSING AND STORAGE ON CHANGES IN THE AMINO ACID COMPOSITION OF CANNED ORANGE JUICE

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It was reported previously that excessive heating of orange juice caused the partial destruction of several amino acids including alanine, serine, aspartic acid, glutamic acid, cysteine, and the tripeptide, glutathione. Therefore, it was of interest to determine whether the free amino acid content of orange juice would be affected significantly under processing and storage conditions more closely resembling those actually encountered.

Approximately 50 gallons of a homogeneous batch of commercially reamed and deaerated California Valencia orange juice (Brix:acid ratio=13:6) were filled into approximately 1500 enamel-lined 6-oz. cans under an inert gas and transported to the laboratory at 32°F. in a refrigerated truck. No significant flavor change was observed during storage for 48 hours at this temperature. The juice was processed in a hot-water bath with vigorous agitation as follows:

Batch	Processing temperature= °F.	Processing time= Minutes 1	2	4	8	16
A	160			x		
B	180			x	x	x
C	190		x	x	x	
D	200	x	x	x		
E	210			x		

Approximately one minute each was required to raise the juice to the processing temperature and lower it to the temperature of the cooling water. Samples were stored at 0°, 40°, 70°, and 100°F. and are being removed periodically for measurement of changes in flavor, color, and cloud stability, as well as for the estimation of changes in the aspartic acid, glutamic acid, alanine, serine, arginine, proline, and gamma-aminobutyric acid content of the juice.

The control sample, the eleven lots of heat-processed juice, and a sample of commercially pasteurized juice from the same lot were analyzed at zero storage time to estimate the effects of the various processing conditions. Minimal changes in all variables occurred during processing. However, optimum flavor retention was observed at processing temperatures between 180° and 200°F. Analyses of samples after three months' storage at 40°, 70°, and 100°F. are now in progress. Storage studies will be continued for an additional period of three months.

PROGRESS REPORT ON TEXAS GRAPEFRUIT RESEARCH

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The Weslaco Laboratory activities are devoted to research on the utilization of fruit and vegetable products. The work on citrus fruit is the major project, under the title "Development of improved methods of processing Texas pink and red grapefruit to obtain products of optimum color and flavor for military and civilian use." The emphasis is on colored grapefruit because 80% of the new plantings of citrus are colored grapefruit.

Work on concentrated juice will be postponed until fruit is more abundant. Present emphasis is on the improvement of single-strength juice of Ruby red grapefruit by pulp-fortification. Methods of color evaluation are necessary to evaluate the products and to provide information on the development and disappearance of the color in the fruit.

Studies previously reported show color development in relation to seasonal maturity of the fruit. This report indicates that the color maximum is reached before optimum maturity of the fruit. There is, therefore, a relatively short processing period for obtaining the most color. Continued work the past two seasons has confirmed this seasonal change.

The content of carotenoid pigments has been found to correlate well with reflectance measurements of the blended edible portion of the fruit. The correlation is expressed by comparing the a/b ratio, i.e. redness/yellowness ratio obtained from the Gardner Automatic Color Difference Meter, with the ratio of lycopene/2 times the concentration of carotene.

The method of measuring the pigments consists of removing most water soluble material from the blended fruit by mixing with an equal volume of methyl alcohol and filtering. The pigments are then extracted by blending the colored pulp on the filter pad with a 50-50 mixture of acetone and hexane. The extract is washed to remove the acetone and dried with sodium sulfate. The light transmittancy is determined at 455 and 505 millimicrons. The apparent lycopene and carotene values are calculated by simultaneous equations according to the procedure outlined by Comar and Zscheile.

According to unpublished correspondence from Dr. Curl, Western Utilization Research Branch, mature red grapefruit contained 31.0% carotenes (mainly beta with lesser amounts of zeta and gamma), 53.9% lycopene, 7.8% colorless polyenes, and 6.7% carotenoids. These results would indicate that the carotene and lycopene values obtained by use of simultaneous equations after measuring absorbtion at 455 and 505 m μ. are slightly high. Previous analyses involved separation of the pigments on a column of magnesium oxide and filter aid, and elution. This chromatographic procedure yielded results which averaged about 40% lower for lycopene and 20% lower for carotene

than values obtained on the extract without separation of the pigments. A correlation between the reflectance measurements and pigments determined by use of chromatography was not readily apparent. The discrepancy between the two methods of pigment analysis is now being studied by the Analytical and Physical Chemistry Section of Southern Utilization Research Branch in New Orleans.

Data have been accumulated which show a direct relationship between color and pulp content of the juice. In-plant work this season is expected to provide necessary information for the addition and suspension of maximum amounts of colored pulp for commercial preparation of single-strength juice. In addition to increased color there will be an increase in provitamin A and an increase in yield.

Seasonal color measurements by reflectance and pigment analysis have indicated a slightly greater color from groves on sandy loam than on the heavier clay soils.

A more intensive investigation of color variation, development, and decline in Ruby Red grapefruit is in progress. In cooperation with the Citrus Rootstock Investigations Section of the Horticultural and Crops Research Branch of Agricultural Research Service and Branch 15, Texas Agricultural Experiment Station, studies are in progress on the variations caused by growing Ruby Red budwood on different rootstocks.

Work on color development in Red blush, Thompson pink, and Marsh seedless grapefruit is in progress. Pigment analyses of the three types were started in June and are being made at two-week intervals. The pigment content of the white fruit has remained nearly constant. The lycopene content of the red and pink fruit reached a maximum near the end of September and declined quite rapidly. The carotene content continued to increase for at least two more months. It was found that during the early part of the sampling season incubation of chunks of the edible portion caused an increase in the amount of pigment. The mechanism of the increase has not yet been explained.

VAPOR PHASE CHROMATOGRAPHY--A NEW TECHNIQUE

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Distillation is the method normally employed in the analysis and separation of volatile materials. The recent development of gas-liquid partition chromatography (GLPC) may well revolutionize analytical chemistry. The term chromatography has become a convenient generic description for analytical separations depending upon differential sorption or solution of the components of a flowing mixture with respect to a stationary solid medium, and several variations of this method have become established for different purposes.

GLPC differs from conventional chromatography in that the adsorbent is replaced by a relatively non-volatile liquid, e.g. silicone fluid, retained on a solid support, such as Celite 545, while the mobile phase is no longer a liquid but an inert gas, e.g. helium. Volatile components of a mixture are eluted from such a column at rates proportional to their respective vapor pressures over the column liquid. Other factors influence the elution rates (i.e., the degree of separation), such as van der Waal's forces, hydrogen bonding, etc. The process of separation is thus clearly analogous to extractive distillation, and the efficiency of the column may be expressed in terms of the number of theoretical plates. On this basis, efficiencies as high as 1000 theoretical plates in a four-foot column are possible. By choice of an appropriate material for the stationary phase it is possible to influence the differential vapor pressure between components in such a manner that closely boiling or azeotrope-forming compounds may be readily separated.

Other advantages of GLPC are its speed, convenient size, economy of sample material, and durability. Very often little more than 15 minutes are needed for the complete separation of a complex mixture. Extremely small (ca. 0.02 ml.) quantities of a mixture are required for an analysis, the lower limit being set only by the sensitivity of the detecting device and not by the column. After complete elution of one sample, a partition column may be immediately used again for the next analysis. No cleaning is required. Thus, provided the stationary phase is stable and non-volatile under the conditions of operation, one column can be employed for an indefinite number of analyses.

If separation is complete, each eluted substance leaves the column as a discrete band, and with use of a thermal conductivity cell as a detecting

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1/ Employed by Lemon Products Advisory Board under Memorandum of Understanding.

device, each substance gives a concentration curve or elution "peak" on the recorder, provided only that its thermal conductivity differs from that of the carrier gas stream. Two substances with similar conductivities will, if resolved, give separate "peaks" which are easily distinguishable.

GLPC is applicable to production control, quantitative and qualitative detection of impurities in a product, separation of components in a natural product, detection of reaction products on completion of a chemical reaction, chemical kinetics, and a host of other most useful applications.